Pharmaceutical BIOPROCESSING

Electrical stimuli in stem cell production and differentiation: an important factor?

"...electric stimulation will play an important part in the production of preconditioned stem cells for tissue regeneration, especially for those in which electric field already played a role in their embryonic formation or tissue function."

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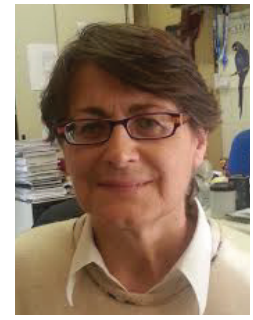

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Stem cells represent the most promising and innovative approach for tissue engineering and regenerative medicine as well as for pharmacological research [1]. In fact, stem cells and their differentiated progeny have already been used to replace lost or injured cells, as, for example, in the case of severe burns or corneal injury [2]. Moreover, they offer multiple advantages in the pharmaceutical pipeline of drug discovery and development and are important tools for functional gene discovery, drug discovery and toxicology screenings. In fact, they offer the possibility of testing molecules for *in vitro* induced differentiation, performing lineage selection or specific cellular assays and testing highly polymorphic variants of metabolic genes for toxicological assays [3].

In the case of adult stem cells, their isolation from a variety of somatic tissues is the first step for their production/expansion and culture. The major problem associated with this step is that, so far, no marker for their unequivocal identification and isolation has been found, and thus a combination of molecules preferentially expressed on these cells, but shared by nonstem cells, must be used [4,5]. Moreover, the number of stem cells is generally low, especially in certain organs/tissues, for example, myocardium or nervous tissue. In all cases, the fact that stem cells are localized in specific sites, the so-called niches [6], can help/direct researchers in finding them. The niche has an essential role in maintaining stem cells, providing signals for their survival and at the same time for their commitment, in other words, their propensity to differentiate toward a specific cell lineage. It is thus clear that there are different adult stem cells depending on the tissue of origin and degree of multipotency. Last, but not least, in 2006 the production of artificial stem cells, the so-called induced pluripotent stem cells, which are obtained by directly reprogramming somatic cells by means of the ectopic expression of four embryonic transcription factors, was reported for the first time [7] and has opened to a new era of both clinical and research applications. The generation of disease-specific induced pluripotent stem cell lines is an important tool for the research and development of strategies to correct genetic defects, as already demonstrated for familial dysautonomia [8].

The use of stem cells as therapeutic tool has been extensively evaluated mainly for cell transplantation and tissue regeneration, and secondarily also for cell-based drug delivery and immunotherapy [1,2].

Maintenance of stem cell proliferation and multipotency during *in vitro* cell expansion are key factors for accurate therapeutic strategies, together with the need to direct the differentiation process, the latter also for the purpose of avoiding the risk of neoplastic transformation and tumorigenesis [9]. The design and development of production systems for the generation of reliable stem cells

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is still an intricate problem. Growth factor-directed differentiation offers good chances for pharmacological driving of conditioning, but strategies for the commitment/differentiation of the cells are still largely empirical and based mainly on supposed modifications of cellular biochemical pathways able to influence genomic expression and phenotypic definitions. These approaches have solid rational bases but consider only some part of the whole series of factors that are involved in such a complex process such as cell conditioning. The pharmacological conditioning of stem cells based on receptor agonists or antagonists has not yet provided the optimal requirements for the production of reliable and safe stem cells. *In vitro* cell commitment/differentiation has been mainly approached by the application of exogenous biochemical factors. However, physical stimuli also contribute to shape the microenvironments, from which the stem cells are derived (the embryonic layer and the niche) or wherein they should be transplanted [10]. Indeed, natural endogenous electric signals have been shown to be important in many aspects of cell life, in other words, proliferation, lineage commitment, differentiation, migration, survival and tissue regeneration [11]. By using electric stimulation, researchers would exploit what happens physiologically in nature. In particular, electric stimulation should be adopted, especially in the case of tissues that are physiologically prone to electric stimuli generation and propagation or that are influenced by electrical fields (ES), such as nervous tissue, skeletal muscle and myocardium [12–14]. The rational basis for the adoption of the correct electric stimulation to stem cells is still largely unknown, since the specific cellular events that occur after the exposure to electric or magnetic fields are complex and require further deep investigations.

> "...electrical stimulation can induce a variety of responses, such as cytoskeleton rearrangements, migration, proliferation and differentiation. In these processes, the conformation of culture plate surface and its conductivity during electrical fields might play important roles."

Transmembrane signaling and gene expression for structural and signaling proteins respond to biophysical stimuli, but these processes vary in different cell types and, moreover, their quantitative effects seem dependent sometimes on the duration of the stimuli, sometimes on their intensity and sometimes on the scheme of stimulation adopted. Notwithstanding, electrical field stimulations at different frequencies, intensity and duration were reported to upregulate tissue-specific gene and voltage-dependent channel expression and to display marked effects of precommitment, especially for neuron and cardiomyocytes [12,15].

However, bibliographic data, taken together, if on one side indicate that the use of biophysical stimuli such as ES is very promising, on the other side underline that much on the biology of stem cells has still to be fully elucidated and that some important variables are missing before standard protocols might be routinely adopted. More efforts should be directed in this area.

Electrical conditioning represents a possible strategy for the improvement of stem cell ability to engraft and reconstitute the appropriate tissue [11]. In this case, the receiving microenvironment plays a crucial role. For example, cardiac lesions due to postischemic stress or tissue necrosis negatively influence the success of the implant. Thus, an appropriate preconditioning of the cells should provide them with resistance to the insults of the host tissue and warrant the survival of transplanted stem cells, their colonization ability in specific areas and the replacement of inactive tissue with differentiated and functionally active somatic cells. Kim and colleagues have proved that ES induces a cytoprotective effect on cardiac stem cells through the activation of the AKT/FAK/CTGF signaling pathway [16]. *In vivo in situ* electrostimulation seems to be able to cause tissue regeneration in the zone of infarcted myocardium, together with a correct remodeling of angiogenesis [17]. These results, among others, underline that the ES approach has big potentiality in stem cell transplant, since it is effective on multiple synergic steps of stem cell tissue regeneration, such as enhancement of cell proliferation, differentiation and survival in the distressing host microenvironment. We have shown that electrically stimulated adult stem cells displayed a transcriptional profile more closely related to that of neonatal cardiomyocytes, thus offering an economic technique for translational applications for heart tissue engineering and regeneration, not requiring expensive exogenous bioactive molecules [15]. We have also shown, as have other authors, that electrical stimulation can induce a variety of responses, such as cytoskeleton rearrangements, migration, proliferation and differentiation. In these processes, the conformation of culture plate surface and its conductivity during ES might play important roles [18,19]. In fact, substrate conductivity together with the material used for its production is able to affect cell proliferation, differentiation and orientation of myoblasts. We think that the science of the conductive materials will provide interesting insights on the manipulation of stem cells.

Furthermore, an intriguing area of ES application is the development of scaffolds for the generation of *in vitro* 3D tissues such as myocardium. Some authors

have indicated that ES plays an important role in directing the plasticity of the scaffold colonization [20], thus opening new frontiers in the production of economic artificial tissues without the use of soluble factors to be used for drug discovery and development.

In conclusion, electric stimulation will play an important part in the production of preconditioned stem cells for tissue regeneration, especially for those in which electric field already played a role in their embryonic formation or tissue function. The large variability among ES protocols described in the literature, together with the lack of information on the influence of the materials used as substrate for stimulation, makes ES application still an empiric science. Furthermore, the improvement in knowledge of the basic biology and biochemistry of stem cells will provide precious insights for the final definition of tissue-specific ES protocols.

Financial & competing interests disclosure

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References

1 McGivern JV, Ebert AD. Exploiting pluripotent stem cell technology for drug discovery, screening, safety, and toxicology assessments. *Adv. Drug Deliv. Rev.* 69C–70C, 170–178 (2014).

2 Jackson WM, Nesti LJ, Tuan RS. Concise review: clinical translation of wound healing therapies based on mesenchymal stem cells. *Stem Cells Transl. Med.* 1(1), 44–50 (2012).

3 Odawara A, Saitoh Y, Alhebshi AH, Gotoh M, Suzuki I. Long-term electrophysiological activity and pharmacological response of a human induced pluripotent stem cell-derived neuron and astrocyte co-culture. *Biochem. Biophys. Res. Commun.* 443(4), 1176–1181 (2014).

4 Lv FJ, Tuan RS, Cheung KM, Leung VY. Concise review: the surface markers and identity of human mesenchymal stem cells. *Stem Cells* 32(6), 1408–1419 (2014).

5 Zamperone A, Pietronave S, Merlin S *et al.* Isolation and characterization of a spontaneously immortalized multipotent mesenchymal cell line derived from mouse subcutaneous adipose tissue. *Stem Cells Dev.* 22(21), 2873–2884 (2013).

6 Rezza A, Sennett R, Rendl M. Adult stem cell niches: cellular and molecular components. *Curr. Top. Dev. Biol.* 107, 333–372 (2014).

7 Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126(4), 663–676 (2006).

8 Lee G, Papapetrou EP, Kim H *et al.* Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. *Nature* 461(7262), 402–406 (2009).

9 Shiras A, Chettiar ST, Shepal V, Rajendran G, Prasad GR, Shastry P. Spontaneous transformation of human adult nontumorigenic stem cells to cancer stem cells is driven by genomic instability in a human model of glioblastoma. *Stem Cells* 25(6), 1478–1489 (2007).

10 Pagliari S, Vilela-Silva AC, Forte G *et al.* Cooperation of biological and mechanical signals in cardiac progenitor cell differentiation. *Adv. Mater.* 23(4), 514–518 (2011).

11 Cui H, Tang L. Stem cell lineage commitment by electrical fields and the potential application in drug discovery. *Curr. Drug Metab.* 14(3), 272–278 (2013).

12 Liu HH, Xiang Y, Yan TB, Tan ZM, Li SH, He XK. Functional electrical stimulation increases neural stem/progenitor cell proliferation and neurogenesis in the subventricular zone of rats with stroke. *Chin. Med. J. (Engl.)* 126(12), 2361–2367 (2013).

13 Flaibani M, Boldrin L, Cimetta E, Piccoli M, De Coppi P, Elvassore N. Muscle differentiation and myotubes alignment is influenced by micropatterned surfaces and exogenous electrical stimulation. *Tissue Eng. Part A* 15(9), 2447–2457 (2009).

14 Hirt MN, Boeddinghaus J, Mitchell A *et al.* Functional improvement and maturation of rat and human engineered heart tissue by chronic electrical stimulation. *J. Mol. Cell. Cardiol.* 74, 151–161 (2014).

15 Pietronave S, Zamperone A, Oltolina F *et al.* Monophasic and biphasic electrical stimulation induces a precardiac differentiation in progenitor cells isolated from human heart. *Stem Cells Dev.* 23(8), 888–898 (2014).

16 Kim SW, Kim HW, Huang W *et al.* Cardiac stem cells with electrical stimulation improve ischaemic heart function through regulation of connective tissue growth factor and miR-378. *Cardiovasc. Res.* 100(2), 241–251 (2013).

17 Spadaccio C, Rainer A, De Marco F *et al. In situ* electrostimulation drives a regenerative shift in the zone of infarcted myocardium. *Cell Transplant.* 22(3), 493–503 (2013).

18 Thrivikraman G, Mallik PK, Basu B. Substrate conductivity dependent modulation of cell proliferation and differentiation *in vitro*. *Biomaterials* 34(29), 7073–7085 (2013).

19 Tandon N, Marsano A, Maidhof R, Wan L, Park H, Vunjak-Novakovic G. Optimization of electrical stimulation parameters for cardiac tissue engineering. *J. Tissue Eng. Regen. Med.* 5, e115–e125 (2011).

20 Miklas JW, Nunes SS, Sofla A *et al.* Bioreactor for modulation of cardiac microtissue phenotype by combined static stretch and electrical stimulation. *Biofabrication* 6(2), 024113 (2014).